# Expression and prognostic value of brain and acute leukemia, cytoplasmic in meningiomas

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*To the Editor:* Meningiomas are the most common central nervous system neoplasms.[1] The World Health Organization (WHO) 2016 classification system classifies meningiomas into three grades: grade I (benign), grade II (atypical), and grade III (anaplastic/malignant) meningioma.[1] Benign meningiomas are usually associated with favorable prognosis; however, higher grade (WHO grades II and III) menigiomas are more aggressive, resulting in less favorable outcome.[1]

Taking advantage of the DNA microarray high throughput screening in identifying leads of molecular and pathological mechanism associated with the phenotypes of meningiomas,[2-5] we previously reported that over 300 genes are differentially expressed between WHO grade I and grade II/III meningiomas.[6] One particular molecule among the up-regulated genes, brain and acute leukemia, cytoplasmic (*BAALC*), is expressed at markedly different levels, a 4.759-fold change, between high-grade and low-grade meningiomas. High *BAALC* gene expression has been associated with poor prognosis in acute myeloid leukemia.[7] To thoroughly determine the potential of *BAALC* as a prognostic marker for high-grade meningiomas, we focused the present study on *BAALC*.

This study included 82 meningioma patients who had undergone surgery at Sir Run Run Shaw Hospital between 1999 and 2015. Of them, 39, 34, and 9 patients were determined as WHO grade I (25 meningothelial, 2 fibrous, 5 angiomatous, 4 microcystic, 2 secretory, and 1 metaplastic), grade II (33 atypical and 1 clear cell), and grade III (6 anaplastic, 1 papillary, and 2 rhabdoid), respectively [Supplementary Table 1, http://links.lww.com/CM9/A78]. The patients were divided into two groups: low-grade meningiomas (grade I) and high-grade meningiomas (grades II and III). Additional parameters were then used to sub-divide the cases into different groups for the following analysis [Supplementary Table 2, http://links.lww.com/CM9/A78].

Archival tissue blocks were used for histopathologic and immunohistochemical (IHC) analysis. EnVision system was used for IHC processing and staining (DAKO, Carpinteria, CA, USA), and the IHC procedures were performed according to the manufacturer's protocol. The primary antibodies with the following dilutions were used: *BAALC* (rabbit polyclonal, 1:150; ProteinTech, Wuhan, China), progesterone receptor (PR) mouse monoclonal, 1:200; DakoCytomation, Glostrup, Denmark), Ki67 (MIB1) (mouse monoclonal, 1:100; DakoCytomation). Secondary antibodies were goat anti-mouse or anti-rabbit (DakoCytomation). The staining of PR and Ki67 (MIB1) was used for nuclear staining. Nuclear labeling index of the two markers was evaluated by counting positive cells in 1000 tumor cells starting from the areas of greatest immunopositivity. Cytoplasmic staining of *BAALC* was scored by a semi-quantitative scoring system based upon the whole staining intensity and percentage of positive cells. The scale for staining intensity is: 0 = negative, 1 = weak, 2 = moderate, 3 = strong, and the scale for percentage score is: 0 = negative, 1 = 0% to 25%, 2 = 26% to 50%, 3 = >50%.

Fresh meningioma tissues were immediately gently homogenized after removal, filtered by cell strainer (100 μm, BD FALCON, USA), cultured in Dulbecco's modified Eagle medium (DMEM) with 10% fetal calf serum (FCS), 100 units/mL ampicillin and 100 ng/mL streptomycin at 37 °C with 5% $CO_2$. All the cell culture media and reagents were from GIBCO (Rockville, Maryland). Recombinant expression plasmid pEGFP-N1-BAALC (YouBao, Changsha, China) was transiently transfected into the primary

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meningioma cells by Lipofectamine 2000 (Thermo Fisher Scientific, USA) according to the manufacturer's protocol.

Transfected cells were seeded in a 96-well culture plate at a density of 2000 cells per well. Ten microliters of cell counting kit-8 (Boster, Wuhan, China) was subsequently added to each well and the cells were incubated for 3 h at 37°C with 5% $CO_2$. The absorbance was measured at 570 nm to calculate the numbers of viable cells in each well. Transfected cells were seeded in a six-well culture plate containing DMEM with 10% fetal bovine serum (FBS) and cultured until they become 90% confluent. The culture medium was removed and two across wounds per well were made by scratching the cell monolayer with pipette tips. The plates were washed twice with phosphate buffer saline to remove cellular debris and added with serum-free DMEM. Time-lapsed images from the same area of the wound were taken under the inverted microscope (Nikon, Japan) at 12, 24, 48, and 72 h after scratching.

The 8-μm transwell 24-well chambers (Costar, Cambridge, MA, USA) were used for primary meningioma cells invasion and migration assays. The invasion assay was performed in the transwell chambers coated with Matrigel (BD Biosciences, San Jose, CA, USA). For the invasion or the migration experiment, cells were re-suspended in DMEM with 1% FCS, and 100 μL cells ($10^6$/mL) were seeded in the upper compartment of the chamber, 600 μL of complete DMEM with 10% FBS was added in the lower chamber, and incubated for 72 h at 37°C with 5% $CO_2$. Cells on the upper side of the chamber filter were removed by cotton swab, and those on the lower side of the filter were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The cells migrated through the membrane or the cells invaded through the layer of matrigel were counted under the inverted microscope (Nikon). General statistical and survival analysis was carried out by using the SPSS Statistics software (Version.19.0; SPSS Inc., Chicago, IL, USA).

We first sought to assess the expression of *BAALC* in various meningioma samples. Immunostaining of the *BAALC* varied from negative to strongly positive in the meningiomas, as shown in Figure 1A–1C. The average relative *BAALC* protein level was 2.51 ± 1.43 in low-grade meningioma, and 4.21 ± 1.51 in high-grade meningioma. The difference was significant between the two groups ($P < 0.001$) [Supplementary Figure 1, http://links.lww.com/CM9/A78]. Significant statistical difference in the immunostaining of *BAALC* was also observed with gender, tumor, and brain invasion [Supplementary Table 2, http://links.lww.com/CM9/A78]. Second, we sought to assess the value of *BAALC* as a prognostic marker for meningiomas. We, therefore, analyzed the correlation between *BAALC* levels and several clinicopathological variables related to the patient outcome in these 82 meningioma patients by disease-specific survival (DSS) and progression-free survival (PFS) analysis. Univariate analysis showed that tumor grade, *BAALC* levels, brain invasion, and necrosis were of prognostic significance with respect to both DSS and PFS [Figure 1D and Supplementary Table 3 and 4, http://links.lww.com/CM9/A78]. The cell proliferation of *BAALC* over-expressing cells was then analyzed [Figure 1E]. These results suggest that over-expression of *BAALC* may promote the proliferation of meningioma cells.

To assess whether *BAALC* affects meningioma cell motility, scratch wound healing assays were performed as described. Time-lapse microscopy images revealed that *BAALC* over-expressing cells migrated to the wound edge at a faster rate compared to the control, covered the wound area at a faster pace compared to control, and closed the wound region in 72 h [Supplementary Figure 2, http://links.lww.com/CM9/A78]; whereas, the control cells transfected with only green fluorescent protein exhibited only minimal or at best partial wound closure activity [Supplementary Figure 2, http://links.lww.com/CM9/A78]. Taken together, these results show that *BAALC* over-expression promotes meningioma cell migration in the scratch wound assay. Alternatively, to confirm that *BAALC* over-expression affects tumor cell migration, transwell migration assays were performed for 72 h as described. Microscopy images of the bottom side of the transwell showed that a significantly higher number of *BAALC* over-expressing meningioma cells migrated through the membrane (1000 cells per field of view) than the control meningioma cells (600 cells per field of view) [Figure 1F]. Thus, *BAALC* over-expression promotes meningioma cell migration in a transwell migration assay. To assess whether *BAALC* over-expression affects tumor cell invasion, transwell invasion assays were performed for 72 h as described. Microscopy images of the bottom side of the transwell showed that a significantly higher number of *BAALC* over-expressing meningioma cells invaded through the matrigel (1300 cells per field of view) than the control meningioma cells (700 cells per field of view) [Figure 1G]. Thus, *BAALC* over-expression promotes meningioma cell invasion in a transwell invasion assay.

In our previous study, we confirmed *BAALC* abnormal over-expressed in high-grade meningiomas at mRNA level.[6] This study analysed its relationship with clinicopathological parameters, and determined its prognostic value for meningiomas. We established that *BAALC* is expressed at higher level in high-grade meningiomas. We also revealed differential expression in primary *vs.* recurrent meningiomas and in patients with or without brain invasion (− *vs.* +). *BAALC* expression has statistically significant prognostic value for both DSS and PFS. All these correlations between *BAALC* expression and clinical pathological parameters suggest a potentially critical role of *BAALC* in malignant progression of meningiomas. Therefore, to uncover the mechanism underlying the correlation, we over-expressed *BAALC* in primary cultured meningioma cells and examined its effect on cell migration and invasion. *BAALC* over-expression promotes the proliferation of meningioma cells, tumor cell migration in a wound-healing assay, the invasion and migration of meningioma cells.

## Declaration of patient consent

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

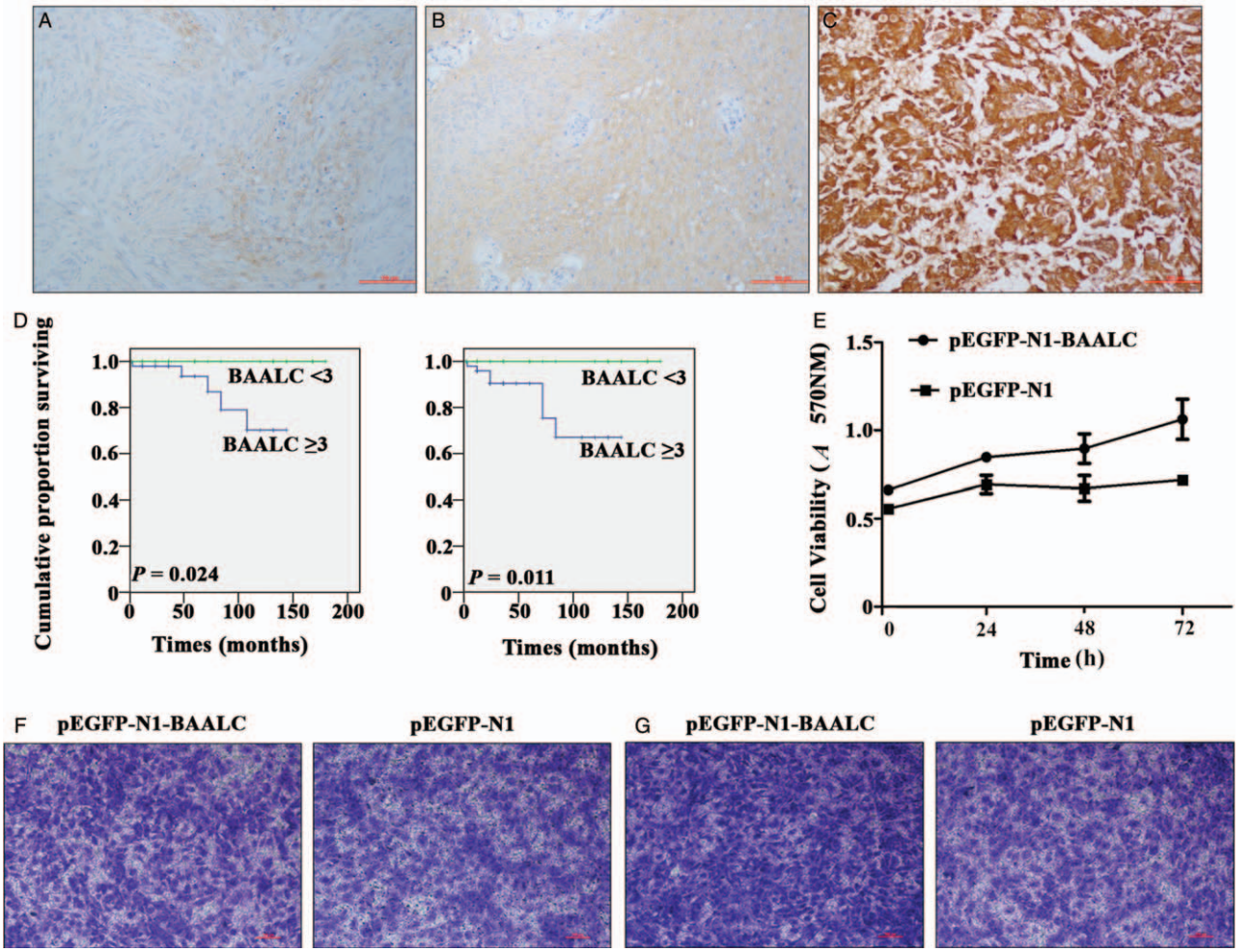


**Figure 1:** (A–C) Representative positive immunostaining of *BAALC* in the three grades of meningiomas: A-grade I, B-grade II, and C-grade III. Simultaneously, the score standards (staining intensity) are also shown: A-score 1, B-score 2, and C-score 3. Horseradish peroxidase labeled EnVision immunostaining with hematoxylin counterstaining. (Orginal magnification for each panel: ×200). (D) Survival analysis. Kaplan-Meier comparisons of DSS and PFS survival for *BAALC* (low *vs.* high). Log rank test *P* values are listed for each parameter. *BAALC* was showed sufficiently prognostic value for DSS or PFS detected by IHC and scored by semi-quantitative scoring system. (E) Cell proliferation analysis. Over-expression of *BAALC* may promote the proliferation of meningioma cells. (F) Transwell migration assay. *BAALC* over-expression promotes meningioma cell migration. (G) Transwell invasion assay. After transfection of fusion protein pEGFP-N1-BAALC and empty plasmid pEGFP-N1, 72 h later, the invasion ability of meningioma cells was observed. *BAALC* over-expression promotes meningioma cell invasion. BAALC: Brain and acute leukemia, cytoplasmic; DSS: Disease-specific survival; IHC: immunohistochemical; PFS: Progression-free survival.


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## Conflicts of interest

None.

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